# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT



(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>12322720/EJH/ar | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416). |
|---|---|---|
| International Application No.<br>**PCT/AU2003/001111** | International Filing Date *(day/month/year)*<br>29 August 2003 | Priority Date *(day/month/year)*<br>30 August 2002 |

International Patent Classification (IPC) or national classification and IPC

**Int. Cl.**[7] C12N 15/53

Applicant

INTERNATIONAL FLOWER DEVELOPMENTS PTY. LTD et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

| Date of submission of the demand<br>15 March 2004 | Date of completion of the report<br>20 December 2004 |
|---|---|
| Name and mailing address of the IPEA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | Authorized Officer<br>**JAMIE TURNER**<br>Telephone No. (02) 6283 2071 |

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
**PCT/AU2003/001111**

## I. Basis of the report

1. With regard to the **elements** of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-133,** as originally filed,
  - pages , filed with the demand,
  - pages , received on with the letter of
- [X] the claims, pages , as originally filed,
  - pages , as amended (together with any statement) under Article 19,
  - pages , filed with the demand,
  - pages **134-137,** received on **30 September 2004** with the letter of **30 September 2004**
- [X] the drawings, pages **1/53-53/53,** as originally filed,
  - pages , filed with the demand,
  - pages , received on with the letter of
- [X] the sequence listing part of the description:
  - pages **1-50,** as originally filed
  - pages , filed with the demand
  - pages , received on with the letter of

2. With regard to the **language,** all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [X] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [X] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Claims 23-24 | YES |
| | Claims 1-22, 25-27 | NO |
| Inventive step (IS) | Claims 23-24 | YES |
| | Claims 1-22, 25-27 | NO |
| Industrial applicability (IA) | Claims 1-27 | YES |
| | Claims | NO |

2. Citations and explanations (Rule 70.7)

The following citations, first raised in the corresponding International Search Report, are referred to as follows:

D1 - EP 0 632 128
D2 - EP 0 522 880
D3 - WO 2000009720
D4 - WO 1996036716
D5 - WO 1993020206

The invention the subject of the international application resides in nucleic acid molecules (chosen from SEQ ID NO: 9, 11, 13, 15, 17, 26 and 31) encoding a flavonoid 3', 5' hydoxylase (F3'5'H), or nucleic acid molecules which encode polypeptides (chosen from SEQ ID NO: 10, 12, 14, 16, 18, 27 and 32) having F3'5'H activity, wherein expression of said nucleic acid molecule in a rose petal tissue results in detectable levels of delphinidin or delphinidin-based molecules as measured by a chromatographic technique. The invention further resides, *inter alia*, in a construct comprising a promoter linked to said molecule, in a method for producing a transgenic plant comprising stably transforming a cell with said nucleic acid molecule and in a genetically modified plant comprising said nucleic acid molecule. The invention also resides in nucleic acid molecules shown in SEQ ID NO: 5 and 30.

D1 discloses F3'5'H gene from petunia petals as well as a transformed plant containing this gene. The transformed plant has bluer flowers or fruit than the corresponding wild type. The sequence shown at SEQ ID NO: 1 shares 68% identity with SEQ ID NO: 12, 66% identity with SEQ ID NO: 14, 65% identity with SEQ ID NO: 16 and 58% identity with SEQ ID NO: 21 of the international application.

Similarly, D2 discloses a petunia F3'5'H gene and plants transformed with said gene. The sequence shown at Figure 9 shares 65% identity with SEQ ID NO: 16 and the sequence shown at Figure 10 shares 69% identity with SEQ ID NO: 12, 66% identity with SEQ ID NO: 14, 58% identity with SEQ ID NO: 21, 73% identity with SEQ ID NO: 27 and 64% identity with SEQ ID NO: 32 of the international application.

D3 relates, *inter alia*, to petunia F3'5'H genes and to plants transformed with said genes. None of the gene sequences disclosed appears to be highly relevant to the gene sequences defined by the claims of the international application.

D4 relates, *inter alia*, to a plant transformed with a construct comprising a F'3'5'H gene and a DFR gene resulting in altered flower color. As with D3, none of the sequences disclosed has a high degree of similarity with those claimed in the international application.

Continued in Supplemental Box

**Supplemental Box**
(To be used when the space in any of the preceding boxes is not sufficient)

**Continuation of V**

D5 relates, *inter alia*, to petunia F3'5'H and to plants transformed with said gene. The gene sequence shown at Figure 3 shares 68% identity with SEQ ID NO: 12, 67% identity with SEQ ID NO: 14, 65% identity with SEQ ID NO: 16, 58% identity with SEQ ID NO: 21, 72% identity with SEQ ID NO: 27 and 63% identity with SEQ ID NO: 32 of the international application.

While none of the cited art explicitly discloses "rose petal tissue (having) detectable levels of *delphinidin* or *delphinidin based* molecules as measured by a chromatographic technique", it is apparent that this feature is an inherent one; that is, if a F3'5'H transformed rose petal was bluer than the wild-type, said rose petal would inherently fulfil the requirements of this feature. Hence, this feature does not contribute novelty over a prior document which discloses a transformed plant petal which is bluer than the wild-type.

In the light of the disclosures of the above prior art, the invention of the international application can be regarded as the provision of the particular gene sequences, derived from Viola, Salvia, Sollya, Lavandula and Kennedia, in order to transform the host plant to produce rose petals having a detectable level of delphinidin or delphinidin-based molecules.

Clearly, however, the identification and purification of a F3'5'H gene from another, analogous species cannot be regarded as inventive. That is, the skilled person would find the identification of F3'5'H from Viola, say, no more than a matter of routine when starting from the position provided by documents D1-D5 above. In fact, D2 provides explicit instructions with regard to the detection of F3'5'H genes in other plants at page 21, line 48 to page 22, line 2. Further, D5 explicitly describes, at page 34, line 30 to page 36, line 25, a method of screening a variety of plant species for sequences homologous to petunia F3'5'H.

Hence, documents D1-D5 are considered prejudicial to the novelty and inventive step of claims 1-22 and 25-27.

It should be noted that none of the cited art disclosed a sequence with a high degree of similarity to SEQ ID NO: 5 or 30; hence claims 23-24 can be considered novel and inventive.

**CLAIMS:**

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a flavonoid 3', 5' hydroxylase (F3'5'H) wherein the nucleotide sequence encodes an amino acid sequence selected from the list consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:27 and SEQ ID NO:32 or an amino acid sequence having at least about 60% similarity to at least one of the amino acid sequences selected from the list consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:27 and SEQ ID NO:32 wherein expression of said nucleic acid molecule in a rose petal tissue results in detectable levels of *delphinidin or delphinidinbased molecules* as measured by a chromatographic technique.

2. The isolated nucleic acid molecule of Claim 1, comprising a nucleotide sequence selected from the list consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:26 and SEQ ID NO:31 or a nucleotide sequence having at least about 60% identity to at least one of the nucleotide sequences selected from the list consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:26 and SEQ ID NO:31, or a nucleotide sequence capable of hybridizing to at least one of the nucleotide sequences selected from the list consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:26 and SEQ ID NO:31 or a complementary form thereof under low stringency conditions

3. The isolated nucleic acid molecule of Claim 1 or 2 wherein the nucleic acid molecule is derived from a plant selected from *Viola* spp, *Salvia* spp, *Lavandula* spp and *Kennedia* spp.

4. The isolated nucleic acid molecule of Claim 2, wherein the nucleotide sequence comprises an overall percentage of less than or equal to 54% of the nucleotides (i) A, or (ii) T, or (iii) A and T in the third nucleotide position of each codon.

5. A construct comprising a sequence of nucleotides comprising: (i) a promoter which is operable in rose petal tissue and wherein said promoter is operably linked to, (ii) a nucleic acid molecule according to any one of Claims 1 to 4.

6. The construct of Claim 5, wherein said promoter is selected from the group consisting of rose CHS, chrysanthemum CHS and CaMV 35S.

7. A construct of Claim 6 wherein said promoter comprises SEQ ID NO:5 or SEQ ID NO: 30, or a functional equivalent thereof.

8. A method for producing a genetically modified plant capable of synthesizing a F3'5'H, said method comprising stably transforming a cell of a suitable plant with a nucleic acid sequence as defined in any one of Claims 1 to 4, under conditions permitting the eventual expression of said nucleic acid sequence, regenerating the genetically modified plant from the cell and growing said genetically modified plant for a time and under conditions sufficient to permit the expression of the nucleic acid sequence.

9. A method for producing a genetically modified plant with reduced indigenous or existing F3'5'H activity, said method comprising stably transforming a cell of a suitable plant with a nucleic acid molecule as defined in any one of Claims 1 to 4, regenerating a transgenic plant from the cell and where necessary growing said genetically modified plant under conditions sufficient to permit the expression of the nucleic acid.

10. A method for producing a genetically modified flowering plant exhibiting altered inflorescence properties, said method comprising stably transforming a cell of a suitable plant with a nucleic acid sequence as defined in any one of Claims 1 to 4, regenerating a genetically modified plant from the cell and growing said genetically modified plant for a time and under conditions sufficient to permit the expression of the nucleic acid sequence.

11. A genetically modified plant or part thereof or cells therefrom comprising an isolated nucleic acid molecule of any one of Claims 1 to 4.

12. A genetically modified plant or part thereof or cells therefrom comprising an isolated nucleic acid molecule of any one of Claims 1 to 4 or comprising an altered level of expression of a nucleic acid molecule of any one of Claims 1 to 4.

13. The genetically modified plant or part thereof or cells therefrom of any one of Claims 11 to 12, wherein the plant part is selected from the group comprising sepal, bract, petiole, peduncle, ovaries, anthers, flowers, fruits, nuts, roots, stems, leaves, and seeds.

14. The genetically modified plant or part thereof or cells therefrom of Claims 11 to 12, wherein the plant is selected from the group comprising rose, carnation, lisianthus, petunia, lily, pansy, gerbera, chrysanthemum, geranium, *Torenia*, *Begonia*, *Cyclamen*, *Nierembergia*, *Catharanthus*, *Pelargonium*, orchid, grape, apple, *Euphorbia* and *Fuchsia*.

15. The genetically modified plant or part thereof or cells therefrom of Claims 11 to 12, wherein the plant is a rose.

16. Use of an isolated nucleic acid molecule as defined in any one of Claims 1 to 4, in the manufacture of a genetic construct capable of expressing F3'5'H or down-regulating an indigenous F3'5'H in a plant, or altering the level of an indigenous F3'5'H enzyme in a plant.

17. A gene silencing construct comprising an isolated nucleic acid molecule as defined in any one of Claims 1 to 4 or a complex thereof.

18. An extract from a genetically modified plant or part thereof or cells therefrom from any one of Claims 11 to 14.

19. The extract of Claim 18, wherein the extract is a flavouring or food additive or health product or beverage or juice or colouring or dye or paint or tint.

20. A eukaryotic organism carrying a genetic sequence encoding a F3'5'H molecule according to any one of Claims 1 to 4 extrachromasomally in plasmid form.

21. The use of a nucleic acid molecule of any one of Claims 1 to 4 in the manufacture of a genetically modified plant or part thereof or cells therefrom.

22. The genetically modified plant or part thereof or cells therefrom of Claim 21, wherein the genetically modified plant or part thereof or cells therefrom exhibits altered flowers or inflorescence.

23. An isolated nucleic acid molecule comprising SEQ ID NO:5 or a functional equivalent thereof.

24. An isolated nucleic acid molecule comprising SEQ ID NO:30 or a functional equivalent thereof.

25. Use of a nucleic acid molecule of any one of Claims 1 to 4 in the identification of genetic material encoding a F3'5'H.

26. Use of a nucleic acid molecule of any one of Claims 1 to 4 in the amplification and cloning of genetic material encoding a F3'5'H.

27. An isolated F3'5'H encoded by the nucleotide sequence of any one of claims 1 to 4.